Chapter 5

Summary & Conclusion

Summary:

The aim of this study was to investigate the genotoxicity of TiO_2 and ZnO NPs on *in vitro* cultured human peripheral blood lymphocytes. Corroborating with the OECD guideline for the need of adaptations to the Chromosomal aberration test, we proposed to study genotoxicity of NPs keeping in mind their physicochemical and dispersion properties in RPMI-1640 culture media. We chose TiO_2 and ZnO NPs because of their increased occupational and human exposure through consumer products. Since NPs possess unique physicochemical properties, investigation of their cellular interaction becomes indispensable. This interaction is typically assessed *in vitro* due to the need for alternatives to animal experimentation, leading to reduction, refinement and replacement of animal use. The biological activity and toxicity of NPs depend on physicochemical parameters such as particle size, size distribution, agglomeration state, and surface charge among the other properties. Thus, characterization of these properties prior to nano genotoxicological investigation was carried out. Dispersion methods can alter the properties of NPs and therefore to mimic the physiological and *in vitro* conditions we used RPMI-1640 complete growth media for characterization of NPs.

In the present work, dispersion and stability studies were done to ensure better correlation with *in vitro* genotoxicity studies. There is a dire need for laboratory data of NP genotoxicity combined with *in vitro* particokinetics, physicochemical; and DNA binding study of NP. Therefore we tried to correlate genotoxicity of NPs with the stability of nano-form in culture, and suggested a possible mechanism of genotoxicity. Fig 5.1 summarizes the work of the thesis. The results of the present work are summarized as follows:

1. X-ray diffraction analysis revealed characteristic peaks for TiO_2 NPs (mix of Anatase and Rutile) and ZnO NPs. The characteristic peaks of Anatase and Rutile present in the graph confirmed that the TiO_2 nano powder used for analysis was a mixture of Anatase and Rutile. Optical properties were determined by observing the absorption peak specific for both NPs. UV- Visible spectrum for TiO_2 and ZnO NP showed a strong absorbance at 385 nm and 375 nm respectively.

2. The physicochemical parameters evaluated by Borosil Mansingh Survismeter revealed that intermolecular interactions of TiO_2 and ZnO NPs with media are higher as compared to water indicating better dispersion and molecular interaction. Particokinetic studies using values from Survismeter revealed that NPs diffuse and settle in cell culture media as a function of systemic and particle properties such as density, viscosity and particle size. Results of DLS and Zeta potential analysis supported the data obtained by Survismeter and suggested better dispersion of TiO_2 and ZnO NPs in RPMI-1640 complete growth media used for *in vitro* culture.

3. The DNA binding studies revealed electrostatic mode of interaction for TiO_2 and ZnO NPs with human genomic DNA. The changes observed in the UV-visible and fluorescence measurements indicate interaction of the NPs with human genomic DNA by a direct formation of a new complex.

4. Following *in vitro* exposure to TiO_2 and ZnO NPs for 24 hours, the cultures were analyzed by Chromosomal aberration assay and Comet assay. The results obtained by Chromosomal aberration assay show that nano sized TiO_2 and ZnO NPs were able to induce a dose dependent increase in the frequency of CA's in cultured human lymphocytes and showed significant clastogenic activity at 75µM, 125µM concentrations ($p<0.05$). Comet assay revealed significant increase in DNA damage in % DNA intensity and Olive Tail moment at specifically 75µM and 125µM for TiO_2 NPs, whereas for all the three concentrations 25µM, 75µM and 125µM nano-ZnO had a significantly greater % DNA intensity in tail and Olive Tail Moment as compared to control.

Conclusion:

- *In vitro* physicochemical and particokinetic studies revealed better dispersion of TiO_2 and ZnO NPs in media than water, in addition to the influence of media properties on density, viscosity and particle size of NPs
- Extent of dispersion and stability was assessed by Dynamic Light Scattering and Zeta Potential analysis suggesting NPs are not agglomerated and available in 'nano' form in culture media
- DNA binding using UV and Fluorescence titrations suggested good binding affinity of TiO_2 and ZnO NPs with DNA indicating direct mode of genotoxicity
- *In vitro* Chromosome aberration assay and Comet assays were performed at low dose exposure for 24 hours, revealing TiO_2 and ZnO NPs are genotoxic

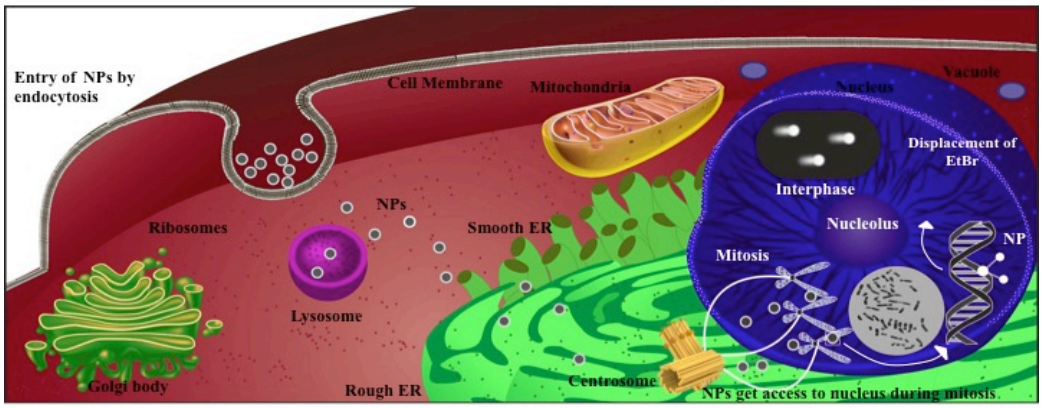

Figure 5.1: Uptake and entry of NPs into nucleus